Prostaglandins, leukotrienes, and the immune response

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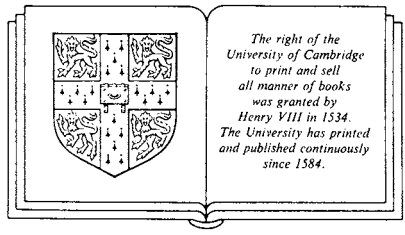

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A brief history and introduction

The products of arachidonic acid metabolism, including the prostaglandins and the leukotrienes, are surprisingly versatile compounds, which participate in an extraordinary variety of normal physiological processes, such as maintaining blood pressure and body temperature, protecting organs from damage caused by disease, traumatic injury and stress, and regulating parturition. In addition, an imbalance in these same metabolites has been implicated in shock and a wide variety of disease states including arthritis, malignancy, and allergic disorders.

In spite of their great importance, most of the lay world and a large portion of the scientific and medical communities, were not introduced to the prostaglandins until the announcement of the Nobel Prize in Medicine for 1982. Swedish chemist Sune Bergström, his colleague Bengt Samuelsson, both of the Karolinska Institute, and British pharmacologist John Vane of Wellcome Research Laboratories received Nobel recognition for their part in determining the structure and the biological role of the prostaglandins. Bergström's pioneering contribution was the discovery that prostaglandins are synthesized *in vivo* from dietary polyunsaturated fatty acids. Along with von Euler, Bergström was able to purify and further characterize the prostaglandins, which had been discovered 20 years earlier. The metabolic fate and disposition of the prostaglandins in the body were determined mainly by Samuelsson and his group. Vane demonstrated the ability of antiinflammatory substances such as aspirin to inhibit prostaglandin synthesis. Bergström, Samuelsson, and Vane also participated in the elucidation of many of the fatty acid derivatives, and the recognition that they often work as antagonistic pairs. One metabolite, for example, lowers blood pressure and another raises it. One dilates bronchi and a second constricts them. One promotes the inflammatory process, and another inhibits it. In 1973, Samuelsson showed that platelets produce thromboxane, which then participates in the

coagulation cascade. Vane identified prostacyclin in 1976, a powerful inhibitor of clotting.

The elucidation of these compounds, however, really began in 1930 with the work of Raphael Kurzrok and Charles C. Lieb, who studied the ability of human semen either to relax or to contract isolated strips of uterine tissue, depending upon whether the woman was sterile or fertile (1). Several years later, Maurice Goldblatt in England and Ulf von Euler in Sweden independently observed a similar phenomenon using human seminal plasma and extracts from sheep seminal vesical glands (2). Believing that the substance he had isolated was a product of the prostate gland, von Euler coined the misnomer *prostaglandin*. More recent work has shown that prostaglandins are not unique to any specific tissue, but instead are synthesized by a wide variety of cell and tissue types.

In the early 1950s, Vogt reported his observations of a highly active, apparently endogenously synthesized biochemical substance in the intestines, which he called *Darmstoff* (3). Several years later Ambache described a highly active lipid material in his experiments with rabbit iris, to which he gave the name *irin* (4). Welsh physiologist V.R. Pickles later described what he thought to be a singular compound, then called *menstrual stimulant*, which displayed the ability to activate powerful contractions of the uterus during menstruation; in 1965, a team of researchers at St. Louis University discovered a substance in the medulla of rabbit kidney, which lowered blood pressure (reviewed in 5, 6). All of these apparently unrelated biological events have since been attributed to the presence and action of the prostaglandins.

Leukotrienes were discovered during the elucidation of a mixture of compounds called the slow-reacting substance of anaphylaxis or SRS-A. SRS-A was first described in 1940 by Kellawey and Trethewie (7) to be a product of immediate-type hypersensitivity. When recovered from perfused guinea pig lung, this isolate was found to constrict smooth muscle tissue more slowly than histamine and, therefore, was named SRS-A. It is possible that Harkavy recognized the presence of a similar substance in the sputum of asthmatic humans as early as 1930 (8). SRS-A was later characterized as a unique "polar lipid" by Brocklehurst (9) and by Strandberg and Uvnas (10), distinct from the family of lipids identified as prostaglandins. Research, however, was greatly hampered by the extremely minute quantities of this material, as well as by substantial degradation of the compound during isolation attempts. The composition of SRS-A was eventually determined, however, by Samuelsson and colleagues, by Morris, Piper, and colleagues, and by Lewis, Austen, and

colleagues to be a mixture of leukotriene C_4 (LTC_4) and its biologically active conversion products, LTD_4 and LTE_4 (11–13). Working from a knowledge of these substances, Radmark et al. definitely established that leukotrienes were synthesized from arachidonic acid, by way of the initial formation of hydroperoxyeicosatetraenoic acid (5-HPETE), then conversion to LTA_4, which is then further metabolized to either LTB_4 or LTC_4 (14).

With the elucidation of the prostaglandins and the leukotrienes as families of distinct, biologically active compounds, has come a great quantity of research activity to describe their natural occurrence and physiological roles. One of the more exciting and significant of these roles is the participation of the prostaglandins and the leukotrienes in the regulation of immune responsiveness. Unfortunately, our understanding to date is incomplete and conflicting data at times are hard to resolve. Because of this, author bias is very evident in the chapters which follow, in terms of the emphasis given to various bits of data, the hypotheses constructed, and the studies relegated to "conflicting data" status.

A BRIEF OVERVIEW OF THE IMMUNE RESPONSE

In the chapters which follow, the immune response will be described to consist of two basic components: the nonspecific and the specific immune response. While the specific immune response is capable of recognition and an amplified secondary response, nonspecific immunity refers to the protective roles of such varied systems as the unbroken skin and phagocytosis, which are not capable of a secondary response. The inflammatory process is a part of nonspecific immunity.

Inflammation is initiated by vasoactive amines such as histamine, serotonin, and the kinin polypeptides. Venular sphincters constrict and capillaries dilate while the kallikrein–kinin system increases vascular permeability and the adherent properties of the venular endothelium. As a result, fluid and fibrinogen leave the permeable vessels creating a fibrin network and thrombin, which contain invading bacteria. The adherent endothelial vessel walls trap phagocytes and allow their emigration into tissues. The kinins also aid in leukotaxis.

Inflammation brings serum into contact with invading microorganisms, aiding in their destruction by such nonspecific compounds as betalysin (lethal to Gram-positive organisms), and the complement cascade (lethal to Gram-negative organisms and a promoter of neutrophil phagocytosis). Complement activation leads to bacteriolysis and

also the release of cleavage products with even greater activity. For example C5a and C3a, cleaved from C3, have glycoprotein components and an affinity for cell membranes (effecting vascular permeability among other things); C3a, C5a, and C567 have powerful chemotactic properties for polymorphonuclear leukocytes (PMN). Finally, inflammation results in the release and circulation of a variety of mediators with strong immunological activity, such as the prostaglandins and leukotrienes, some of which regulate lymphocyte function.

Within minutes of tissue damage or microbial invasion, PMN adhere to blood vessel walls and then emigrate into the tissue. This movement, chemotaxis, is unidirectional with no return of the cells to the circulation, and is facilitated to a large degree by complement. PMN are very active in phagocytosis, a process augmented by opsonins, which include complement and antibody. The intracellular destruction of phagocytized bacteria by PMN is facilitated by a "respiratory burst" of cellular activity, which includes:

1. increased glycolysis and lactate production;
2. a fall in the pH in phagocytic vacuoles;
3. increased O_2 consumption;
4. increased hexose monophosphate shunt activity;
5. increased NADPH and NADH oxidation;
6. increased H_2O_2 and superoxide production;
7. increased membrane lipid synthesis.

If inflammation cannot contain a local infection, invading microorganisms are carried to the regional lymph nodes via the lymphatics. Here, fixed macrophages phagocytize and often succeed in killing organisms where PMN have failed as they contain a repertoire of enzymes that is totally different. The system of fixed macrophages, contained in the spleen, lymph nodes, liver (Kupffer cells), lung (alveolar macrophages), and skin (Langerhans cells) are known collectively as the reticuloendothelial system. These cells exist in enormous numbers (approximately 200 billion are present in spleen, liver, and bone marrow) making the macrophage a central cell in the immune response. Blood-borne macrophages, or monocytes, are capable of phagocytosis and chemotaxis like PMN. Unlike PMN, however, all macrophages can undergo activation after antigen exposure, making them even more efficient in phagocytosis and intracellular killing. Lymphocytes can trigger macrophage activation by means of cellular secretions (lymphokines) and macrophages can likewise influence lymphocyte response via monokines (such as interleukin 1 (IL-1)).

The second major division of the immune reactivity is the specific immune responses of the lymphocyte. The lymphocyte population consists of two separate (more or less) response systems; the T-cell system, which is responsible for cell-mediated immunity and much of immunoregulation, and the B-cell system, which is responsible for antibody production. B and T cells arise from a common bone marrow precursor, but then mature via different pathways. Of the circulating pool of lymphocytes, approximately 80% are T cells and 12–15% are B cells. The remaining lymphocytes do not fall clearly into either category.

When stimulated by antigen, T cells undergo blast transformation then proliferate to form:

1. memory cells, which live as long as 20 years;
2. effector cells capable of a variety of responses including cytotoxicity;
3. regulatory (helper and suppressor) cells, which influence almost every aspect of the immune response, including PMN.

The regulatory activities of these specialized T cells are mediated through the elaboration of lymphokines (such as interleukin 2 and 3 (IL-2, IL-3)).

B cells are responsible for humoral immunity, the hallmark of which is the production of specific antibodies by the plasma cell progeny of activated cells. B cells can secrete five types of antibody, although it appears that only three (IgG, IgM, and IgA) participate in immunity to invading pathogens. The response of these cells and their products is specific and avid, and often carried out together with the products of the complement cascade.

LYMPHOCYTE SUBPOPULATIONS

One of the most important immunological technologies to be developed during the last decade is an ability to identify functional lymphocyte subpopulations on the basis of cell surface differentiation antigens. Monoclonal antibodies to these antigens, raised using the technique of Kohler and Milstein for the production of myeloma–lymphocyte hybrid cell lines (15), have become important tools in identifying and isolating these subpopulations. To date, several extensive series of monoclonal antibodies have been developed primarily to describe lymphocyte subsets (16–18), which are now available commercially. CD3 antibody defines an antigen present on 90–95% of all circulating, peripheral blood T cells, the CD4 antibody identifies 50–60% of T cells, and the CD8 antibody identifies 30–40% of human peripheral blood T cells. Early

Table 1.1. *Representative monoclonal antibodies that are available for the discrimination of specific lymphocyte subpopulations, their specificity, CD designations, and their commercial sources.*

CD group	Example MoAb	Specificity
CD1a	Leu 6,[a] OKT6	Cortical thymocytes, B2M-associated
CD2	OKT11	95% of thymocytes; >90% E-rosette positive lymphocytes
CD3	Leu 4; OKT3	All mature T cells and medullary thymocytes
CD4	Leu 3a,b; OKT4	Helper/inducer subset of T lymphocytes, class II MHC specific cytotoxic cells
CD5	Leu 1; OKT1;[b] T101[c]	All mature T cells and medullary thymocytes; low density on cortical thymocytes
CD8	Leu 2a,b; OKT8	Suppressor subset of T lymphocytes, class I MHC specific cytotoxic cells
CD11b	OKM1	Monocytes/phagocytic cells, some granulocytes and large granular lymphocytes
CD16	Leu 11b	Human NK cells and neutrophils
CD20	B1	All normal (IgG bearing) B lymphocytes
CD25	Tac	T cell specific activation antigen
—	NKH-1[d]	Large granular lymphocytes (LGL) including cells with natural killer activity
—	Leu 7	Medium and large lymphocytes (LGL) including cells with natural killer activity
—	Ia	90% of B lymphocytes and monocytes, 20% of null cells, activated T lymphocytes

[a]The "Leu" designated monoclonal antibodies and B1 are products of Becton-Dickinson.
[b]The OKT designated monoclonal antibodies and Ia are products of Ortho Diagnostics.
[c]T101 monoclonal antibody is a product of Hybritech.
[d]NKH monoclonal antibody is a product of Coulter Immunology.

studies showed that the CD4 antibody labeled the helper T cell subpopulation, and the CD8 antibody labeled the suppressor cells and cytotoxic T cell effectors. More recent studies, however, have shown this distinction to be an oversimplification. For example, the CD4-reactive subpopulation contains at least four separable functional subsets including both helper and suppressor cells (19), and it is now possible to distinguish CD8-positive suppressor cells from CD8-positive cytotoxic cells (20).

In spite of this imprecision in labeling lymphocyte subpopulations, monoclonal antibodies have greatly facilitated our understanding of T–T

and T–B lymphocyte interactions. We now know, for example, that suppressor cells contained within the CD8-positive population require the presence of radiosensitive CD4 lymphocytes in order to function as suppressors. It is also known that CD8 suppression is directed toward CD4-positive helper cells, and not B cells, for example, in the regulation of antibody production. These probes, some of which are summarized in Table 1.1, continue to add to our knowledge of the immune system, each month bringing a multitude of scientific reports on the subject of lymphocyte subpopulations and their interactions.

Recent efforts have been made to standardize the nomenclature of monoclonal antibodies directed against leukocyte surface antigens. Monoclonals from various sources, which show similar reactivity are given a CD (cluster of differentiation) designation. Though not all antibodies have been as yet classified, this ongoing effort and the designations assigned will eventually supersede all the other systems (21). CD nomenclature is, therefore, included in the examples shown in Table 1.1.

IMMUNOLOGIC INTERACTIONS

A major emphasis in immunology today is the study of the interactions of the various components of the immune response. For example, the stimulation of suppressor-T-cell activity can result in altered B-cell activation and a reduction in antibody production. This can alter complement activation and thus PMN function. Likewise, suppressor T cells can adversely affect T-cell response and macrophage function. The products of inflammation are strong stimulators of suppressor cells and thus are very important to both specific and nonspecific immunity.

It is important to remember that, in reality, there is a close interaction of all components of immune responsiveness. Figure 1.1 illustrates some of the ways that the immune response orchestrates the destruction of pathogenic microorganisms. What affects one component of the immune response will directly or indirectly affect them all.

The monocyte/macrophage seems to be a key cell in immunological interactions as a result of its ability to secrete immunoregulatory products. Activation of the monocyte/macrophage results in changes in its secretory functions (Figure 1.2). Some of its secretory products regulate both the monocyte/macrophage and the lymphocyte population. It is now clear that PGE is one of the monocyte/macrophage products, which plays a role in regulating the immune response through its effects on various cellular populations. PGE production also appears to inhibit

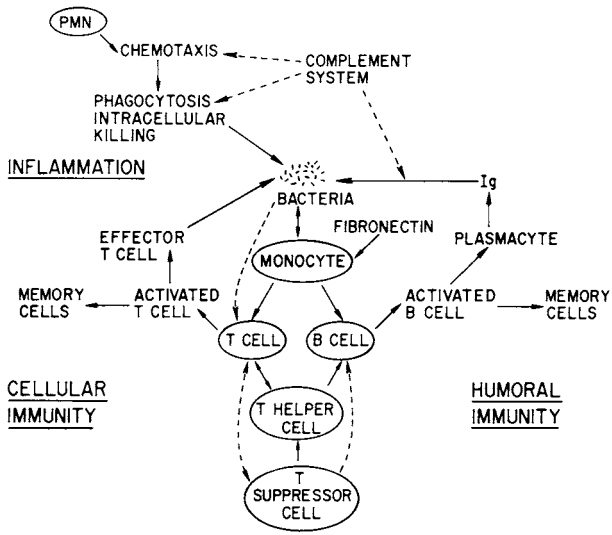

Figure 1.1. A diagrammatic summary of the interactions of the various components of the normal immune response when confronted with bacterial antigens. Each component is dependent on the efficiency of at least one other component. An effective response to a specific pathogen, however, may rely on the particular efficiency of an individual component of the immune response. PMN, polymorphonuclear leukocyte.

further activity of the monocyte/macrophage, including a down-regulation of colony stimulating factor (CSF) production. CSF is necessary to signal precursor cells to proliferate, and its secretion leads to the production of functional inflammatory cells. CSF is known to stimulate precursor stem cells for leukocytes, platelets, and erythrocytes, and to stimulate monocyte/macrophage growth, replication, and function. The production of IL-1 induces T lymphocytes to express cell surface markers and to produce lymphokines, and induces B lymphocytes to produce antibody. IL-1 also stimulates T lymphocytes to produce IL-2. IL-2 promotes the development of functional T-effector lymphocytes, and natural killer (NK) cells, and supports the growth of T cells. As a result of IL-2 stimulation, NK cells secrete gamma interferon. Interferon is also secreted by the activated monocyte/macrophage, and induces these and NK cells to express tumoricidal and bacteriolytic activity (22).

Present and future attempts to manipulate the immune response clinically, therefore, might include the administration or regulation of the production of lymphokines, interferon, or arachidonic acid metabolites.

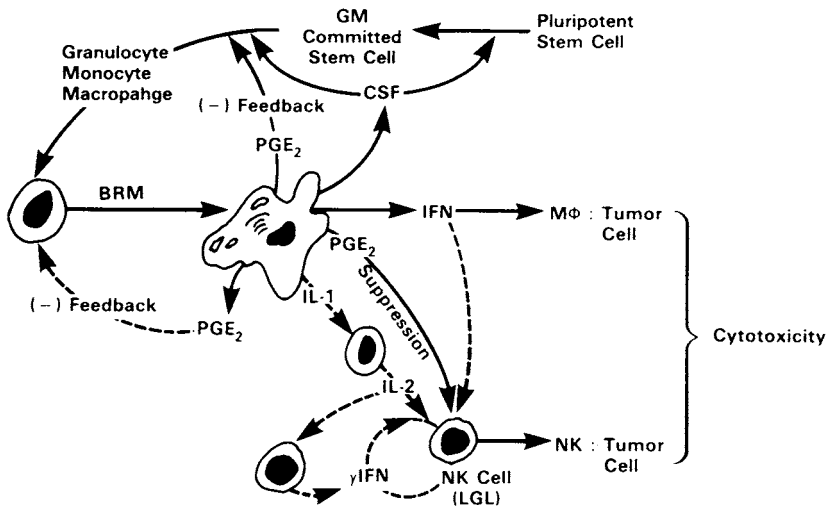

Figure 1.2. The regulation of cellular functions by secretory factors produced by the monocyte/macrophage. GM, granulocyte/monocyte; BRM, biological response modifier; PGE_2, prostaglandin E_2; IFN, interferon; $_{\gamma}$IFN, gamma interferon; M ϕ, macrophage; IL-1, interleukin 1; IL-2, interleukin 2; NK, natural killer cell, LGL, large granular lymphocyte. (Reprinted with permission from Chirigos MA, Schlick E, Ruffmann R: Biological response modifiers: regulation of the cellular immune system. *In*: Gruber D, Walker RI, MacVittie TJ, Conklin JJ (eds.), *The Pathophysiology of Combined Injury and Trauma*. Academic Press, New York. pp. 205–26, 1987.)

Hopes of predictably altering immune reactivity, however, must be based on a clear understanding of the influence and interaction of each of these mediators. In the case of the products of arachidonic acid metabolism, this understanding is still incomplete, as will become evident in the discussions which follow.

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